An in vitro epithelium that bears the mechanobiological hallmarks of living tissue

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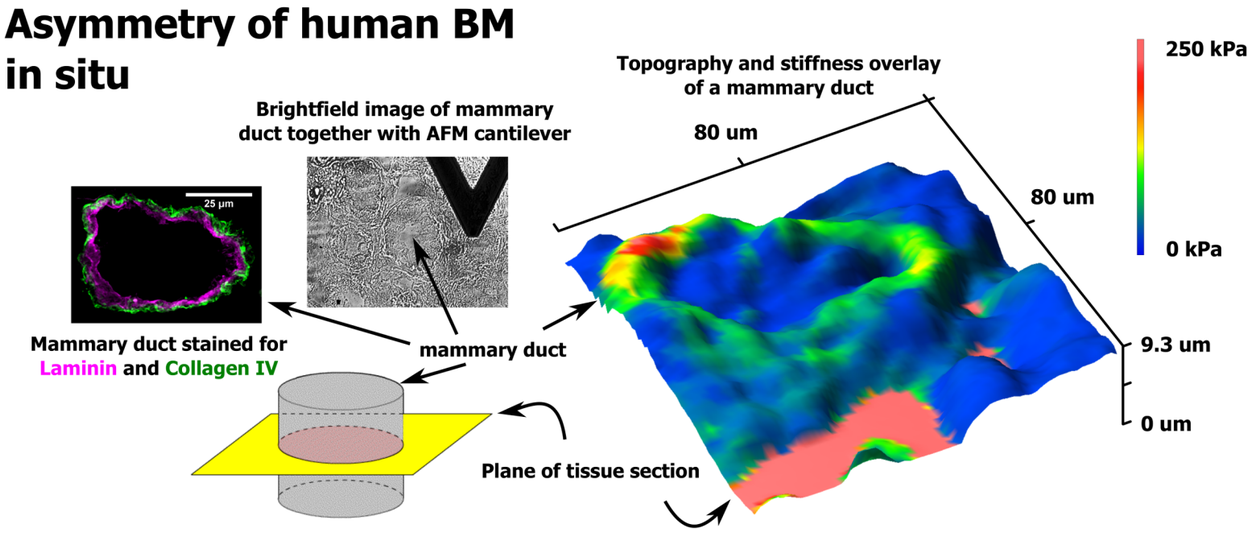
Basement membranes (BM) show high abundance in the human body. By lining endothelial and epithelial lumina (e.g. blood vessels and mammary glands), they provide an interface between populating cells and the underlying connective tissue. Besides constituing a substrate for epithelial and endothelial cells, respectively, these highly conserved structures also work as mechanical barriers against cancer cell propagation[1]. Breast cancer initiation prominently takes place in mammary ducts or lobules. Breaching the basement membrane allows cancer cells to disperse throughout the body and disseminate by forming metastases[2]. Understanding this early process in metastatic cancer progression requires a better insight into the characteristics of both BMs and the evading cancer cells. **The inner limiting membrane (ILM)** extracted from the human eye has turned out to be a valuable tool to bring epithelial cell culturing closer to in vivo conditions. Culturing cells on top of the ILM under hypoxic conditions leads to a significantly softened phenotype which correlates with previous findings in vivo and in situ, postulating 10% of the total cell population within a malignant lesion in human breast to be remarkably soft and hypoxic[3].

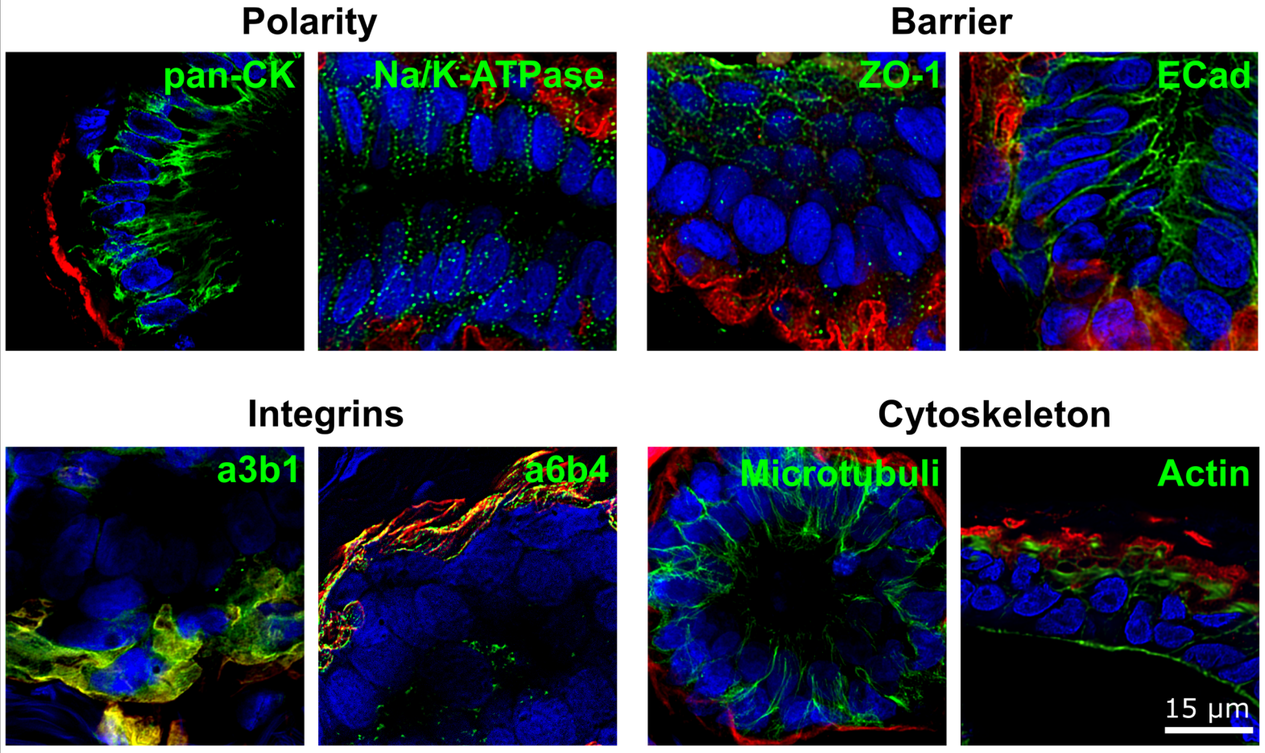
Various microscopy techniques, ranging from mechanical to optical approaches, allow us to gain a deeper insight into BMs as structures fulfilling crucial functions within the human body. Staining for various BM constituents followed by **optical imaging** and **atomic force microscopy** allowed us to asses a multi-facetted understanding of our in vitro tissue model.

Asymmetry of human BM in situ



Combining AFM and fluorescence microscopy we visualize and assess stiffness of BM components in situ using tissue sections. With this approach we show that BMs of human mammary ducts and kidney distal tubules are 30 to 100 kPa stiff which corresponds to the stiffness values of ILM.

BMs and the organization of epithelial architecture in human tissue



Basement membranes line epithelial organs throughout the body and serve as a substrate for epithelial cell layers. Frozen sections of human breast tissue stained for laminin, DAPI and various markers of epithelial polarity, barrier and cytoskeletal components as well as integrins provide us with information about cellular organization.

Conclusion

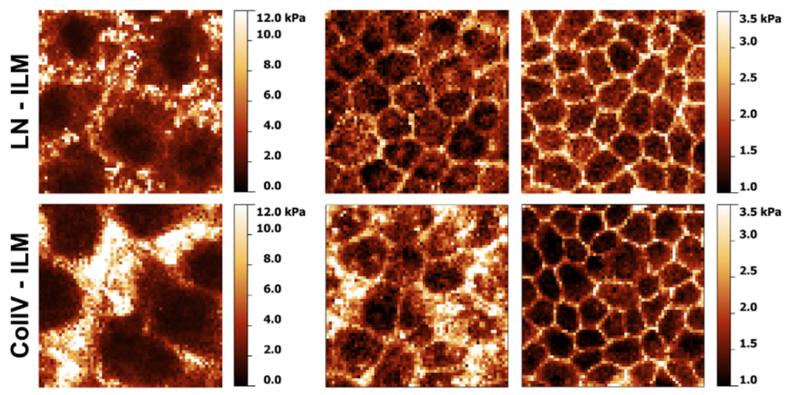
Basement membranes in tissues are organized in distinct epithelial and stromal layers that consist of laminin and collagen IV respectively. Epithelial cells cultured on native BMs exhibit mechano-cellular properties that are distinct from equivalent cells grown on reconstituted BMs (i.e., Matrigel). We show that the mechanical cues can have completely different effects on cell behavior, depending on the structural and mechanical phenotype of the substrate. The direct interactions between the β4-integrin receptor and the ILM laminin that in turn alter intracellular signaling and cytoskeleton reorganization are the essential features of native BM providing physiological microenvironment for epithelia.

REFERENCES

[1] Kalluri, R., Nature Review Cancer 3, 422-433 (2003)
[2] Wirtz et al., Nature Review Cancer 11, 512-522 (2011)
[3] Plodinec et al., Nature Nanotechnology 7, 757-765 (2012)

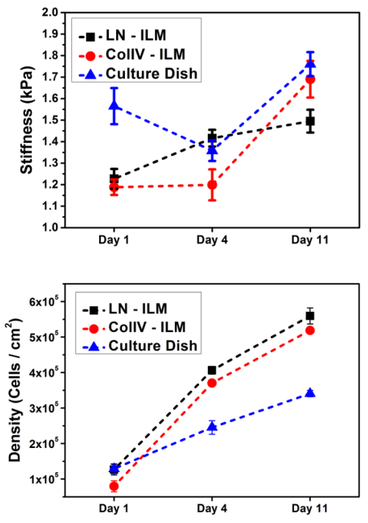
The mechanical phenotype of epithelial cells cultured on a native BM in vitro

TIME-LAPSE STIFFNESS MAPS



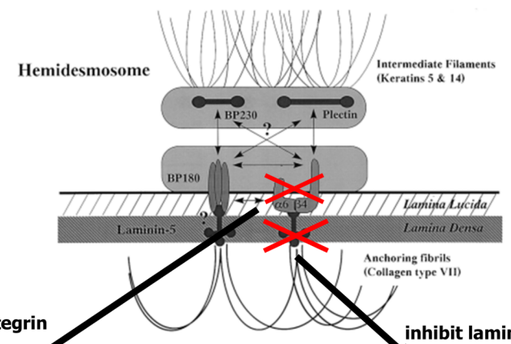
Cell stiffness gradually increases on ILM over time. The mechanophenotype of cells cultured on LN-ILM is steady over time and after 11 days matches the stiffness of cells measured *in situ*[3].

STIFFNESS & DENSITY

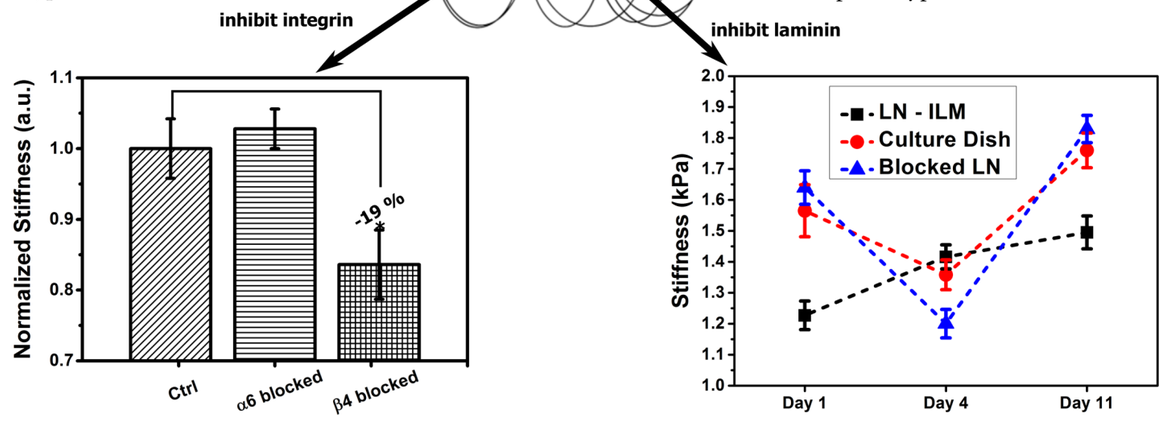


INHIBITING LAMININ AND α6β4 INTEGRINS REVEALS THE FUNCTIONAL ROLE OF BMs FOR EPITHELIAL FORMATION

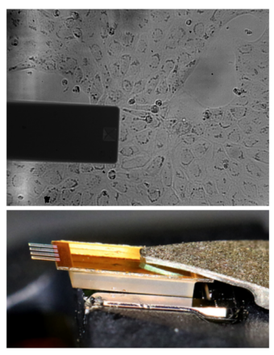
Blocking the β4 integrin leads to a softer mechanophenotype which indicates a distortion of the interaction between ILM and the cytoskeleton. However, blocking integrin α6 does not lead to a significant alteration in phenotype.



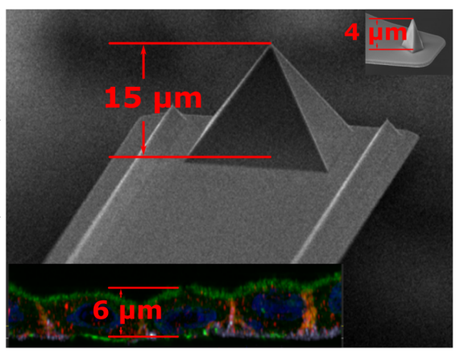
Upon blocking of the LN-ILM with a LN111 polyclonal antibody, cells undergo the same stiffness fluctuations as cells grown on a culture dish. This emphasizes the importance of the laminin/β4 interaction for the physiological phenotype of cells on ILM.

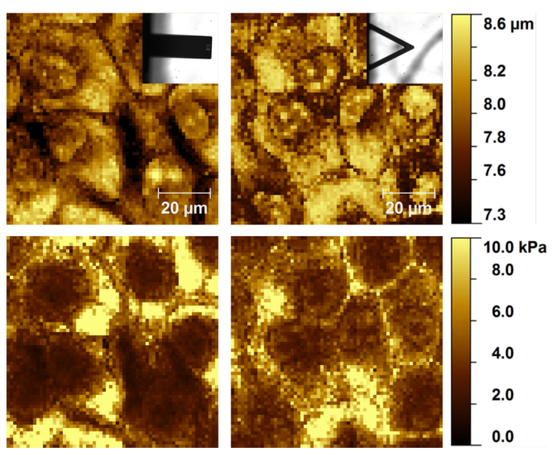


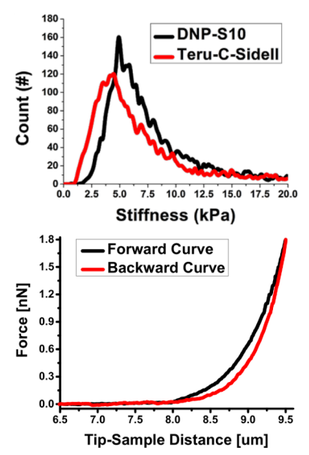
Parallel cantilever arrays perform as well as commercial solution on biological specimens



Tests were conducted on the in vitro cell culture system using rectangular multilever arrays (Type C, side 2, length = 260 µm, width = 100 µm, spring constant = 0.026 N/m) developed by Terunobu Akiyama (de Rooij Lab, EPFL) Overall, we obtained similar quality data from both cantilever types when used forforce spectroscopy under the same measurement conditions







Force spectroscopy (1.8 nN load) was performed on epithelial cells cultured on the native BM. The data in the left column were acquired using a custom made cantilever (Teru-C-SideII) while the data in the right column were collected using a commercially available DNP-S10 (Bruker) cantilever (insets). Yet, we do detect small differences in stiffness, which may be due to deviations in tip shape, geometry and spring constant of each lever.